

# Evaluation of inflammatory markers in patients diagnosed with polycystic ovary syndrome (PCOS)

Inflammatory markers in PCOS

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## Abstract

**Aim:** Polycystic ovary syndrome is one of the crucial problems of women of reproductive ages. The incidence is about 10%. The etiology has not been enlightened, however metabolic disorders and chronic inflammation were accused issues. The neutrophil lymphocyte ratio (NLR) and the platelet lymphocyte ratio (PLR) have been explored for the chronic inflammatory state of the patients. We aimed to evaluate these novel inflammatory markers in patients with PCOS as diagnostic markers.

**Material and Methods:** A prospective case-control study was achieved between June 2020- December 2020. Patients who were diagnosed with PCOS using the Rotterdam consensus criteria, and women who were adjusted for BMI and age as a control group were recruited in the study. Patient characteristics, anthropometric variables, hormonal status, metabolic parameters, high-sensitivity c-reactive protein (hs-CRP), and complete blood parameters were evaluated. **Results:** The numbers of the participants in the PCOS group and the control group were 92 and 85, respectively. Fasting insulin, and HOMA-IR were higher in the PCOS group. HsCRP, NLR, and PLR were higher in the PCOS group, which were statistically significant. The discriminative value of NLR for the presence of PCOS was evaluated using ROC curve analysis. The area under the ROC curve was 0.643 (95% CI: 0.563-0.724,  $p<0.001$ ) for NLR. The optimal cut-off value of NLR for detecting PCOS was  $\geq 2.26$ , with a sensitivity of 57.6 % and a specificity of 62 %.

**Discussion:** Our study revealed that women with PCOS had a higher level of inflammatory markers, and NLR might be a promising diagnostic marker.

## Keywords

PCOS, Inflammation, PLR, NLR, Hs-CRP

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## Introduction

Despite the name, polycystic ovary syndrome (PCOS) seems to be a morphological description, PCOS is a complex metabolic disorder affecting almost 10% of the women in the reproductive period [1]. Diagnostic criteria for ovulatory dysfunction, hyperandrogenism, and ultrasonographic view of the ovaries were constructed [2]. Women with PCOS may suffer from infertility, hirsutism, acne, pregnancy problems as short-term complications, and diabetes mellitus, coronary heart diseases, dyslipidemia, embolism, and psychosocial problems as long-term problems [3, 4]. The adversity of the PCOS is that its pathophysiology has not been precisely clarified. As the accused factors such as insulin resistance and obesity have taken the essential role, nonetheless, women without these features could also experience PCOS [5, 6]. Besides the lack of pathologic pathways, no markers have been found to diagnose or predict PCOS. Inflammation, especially chronic low inflammation, might play a crucial role in the progress of the disease. The fact is that contemporary studies were not able to depict whether the chronic inflammation was a trigger of the disease or a conclusion of the PCOS. However, low chronic inflammation independently of the PCOS could be attributed with diabetes mellitus or cardiovascular disease [7-9]. Adipose tissue is one of the essential sources of that inflammation. Several studies have been achieved to declare that women with PCOS had higher levels of the inflammatory markers like C-reactive protein, tumor necrosis factor- $\alpha$ , interleukin 6, adipon, lipocalin-2 or omentin than in healthy women [10, 11]. During the last decade, physicians have intensified their attention on complete blood count (CBC) parameters, which could be beneficial to expose the inflammatory status of the patients. The neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are considered novel markers of the systemic inflammation. These parameters have been investigated as predictor markers of the diseases associated with chronic inflammation. In the present study, we aimed to expose the association between NLR and PLR with PCOS and the feasibility of these parameters as a diagnostic marker of PCOS.

## Material and Methods

A prospective case-control study was achieved in Bursa Yuksek Ihtisas Training and Research Hospital, University of Health Sciences, between June 2020 and December 2020 in the obstetrics and gynecology department after approval of the local ethics committee. Women were diagnosed with PCOS based on two of the three 2003 Rotterdam consensus criteria [2]. Patients who were admitted to our outpatient clinic with normal menstrual cycles and without hirsutism or hyperandrogenism were included in the control group, which was constructed by matching age and BMI with the PCOS group. All procedures were in agreement with the Helsinki Declaration. Written informed consent was obtained from all participants. Anthropometric variables (age, weight, height, and waist circumference) were measured by a physician who was blinded to the study.

The biochemical evaluation was determined in terms of fasting glucose, fasting insulin, high-sensitivity C-reactive protein (hs-

CRP), total testosterone, and complete blood count. All samples were obtained from the antecubital vein following at least 12 hours of fasting. Serum glucose and hs-CRP were evaluated using an automated analyzer (Abbott Architect C 16000, IL, USA) with its own kits (Abbott Diagnostics, Wiesbaden, Germany). Total-testosterone levels were measured with CMLA (Beckman Coulter Inc., Brea, CA, USA). Serum insulin levels were measured by an automated analyzer (Abbott Architect I2000, IL, USA) using a chemiluminescent microparticle immunoassay (CMLA) with its own kit (Abbott Diagnostics, Wiesbaden, Germany). The Homoeostasis Model Assessment of Insulin Resistance (HOMA-IR), which was used to delineate insulin resistance, was calculated using the formula: fasting insulin [mU/mL]  $\times$  fasting glucose [mg/dL]/ 405. For CBC evaluation, blood samples were obtained into 2 mL EDTA tubes and analyzed using an automated hematology analyzer (Mindray BC-6800 Plus, Shenzhen, China) within 1 hour to avoid time-dependent ultrastructural morphological changes in platelets.

Women who had a concomitant disease that can affect endocrinologic hormonal status, such as Cushing Syndrome, hyperprolactinemia, thyroid disorders, pregnancy, breastfeeding, diabetes mellitus, ovarian or adrenal tumors were excluded from the study. Additionally, patients who had any diseases that can affect CBC parameters such as anemia (Hb < 10.5 gr/dl), leukocytosis (white Blood Cell >12.000  $\mu$ l/ml), thrombocytopenia (Plt < 150.000  $\mu$ l/ml), auto-immune disease, chronic inflammatory disease, or hematological disorders were excluded.

### Statistical Analysis:

We calculated the sample size using a G power calculator (version 3.1). When the power is set to 0.90 and the  $\alpha$ -level is 0.05, the sample size for each group should be at least 70. The demographic, laboratory characteristics, anthropometric measures, and CBC parameters were evaluated. Statistical analysis was performed using the SPSS 24.0 (SPSS Inc., Chicago, USA) software for Windows. The normality test was achieved using the Shapiro-Wilk test. Due to the normality results, the Pearson chi-square test or Mann-Whitney U test was used as appropriate. Correlation analysis of inflammatory markers and other parameters were evaluated using Pearson's correlation analysis. The receiver operating characteristic (ROC) curve was used to determine the diagnostic capacity of inflammatory marker levels for PCOS diagnose. The respective areas under the curve, in which sensitivity was plotted as a function of 1-specificity. All reported confidence interval (CI) values were calculated at the 95% level. An overall p-value of less than 0.05 was considered a statistically significant result.

## Results

One hundred patients were included in each group. After considering the exclusion criteria, the number of patients was 85 in the PCOS group and 92 in the control. The mean values for age, BMI, and waist circumferences were similar, and the comparison of the groups did not differ significantly ( $p > 0.005$ ). Despite there was no significant difference in fasting glucose, insulin, and HOMA-IR values, however insulin and HOMA-IR were higher in the PCOS group nonetheless, fasting glucose values were similar. This could

**Table 1.** Comparison of the demographic and laboratory characteristics of the subjects

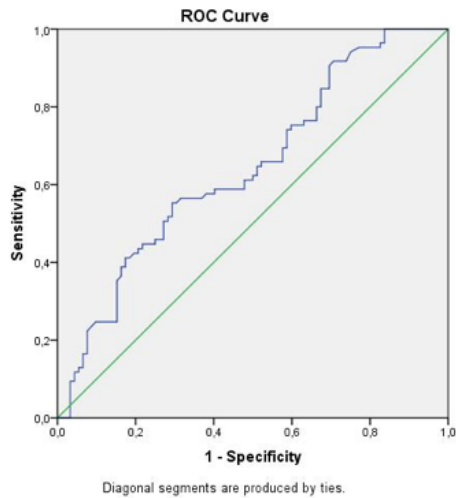
Variables	Control (n=92)	PCOS (n=85)	p
Age, years	26.21 ± 6.35	25.22 ± 6.20	0.300
BMI, kg/m2	24.29 ± 4.62	24.6 ± 5.31	0.646
Waist circumference, cm	77.43 ± 10.37	78.31 ± 11.18	0.587
FBG,mg/dL	93.21 ± 15.97	93.49 ± 12.40	0.898
Insulin, µU/mL	11.85 ± 11.15	17.06 ± 35.63	0.184
HOMA-IR	2.93 ± 3.33	4.32 ± 9.74	0.199
Hs-CRP, mg/L	0.32 ± 0.84	0.81 ± 1.50	0.007
Total-testosterone, ng/ml	1.44±0.65	1.62±0.99	0.155
WBC	7.19±2.17	7.05±1.62	0.627
MPV	8.69±0.93	8.60±0.85	0.539
Neutrofil	4.09±1.33	4.32±1.17	0.005
Lenfosit	1.95±0.62	1.81±0.48	0.103
Platelet	281.46±51.96	296.10±72.56	0.123
Monosit	0.44±0.17	0.43±0.14	0.785
NLR	2.31±1.22	2.80±1.29	0.010
PLR	157.35±56.79	175.32±63.97	0.049

Results are given in mean ± SD. a Independent samples t-test was used. A P value of <0.05 was considered significant (\*). BMI: Body mass index; FBG: Fasting blood glucose; HOMA-IR: Homeostasis model assessment of insulin resistance; hs-CRP: High sensitivity C-reactive protein; PCOS: Polycystic ovary syndrome.

**Table 2.** Correlations between serum NLR and PLR levels and other variables assessed in the control and PCOS groups

	Control				PCOS			
	NLR		PLR		NLR		PLR	
	r	p	r	p	r	p	r	p
Age	-0.082	0.440	-0.179	0.089	-0.017	0.877	0.024	0.834
BMI	-0.164	0.118	-0.080	0.448	0.064	0.562	-0.107	0.328
Waist circumference	-0.017	0.873	-0.012	0.910	0.154	0.160	-0.023	0.835
FBG	0.067	0.528	0.066	0.529	0.054	0.623	0.127	0.248
Insulin	-0.137	0.192	-0.137	0.193	-0.037	0.736	0.089	0.420
HOMA-IR	-0.095	0.365	-0.078	0.457	-0.044	0.690	0.072	0.515
Hs-CRP	0.072	0.495	0.108	0.305	0.172	0.115	0.086	0.434
Total-testosteron	0.024	0.818	0.113	0.285	-0.161	0.141	0.005	0.964

Pearson's correlation analysis was used. r: Pearson's correlation coefficient. A P value of <0.05 was considered significant (\*). BMI: Body mass index



**Figure 1.** Receiver operating characteristic (ROC) curve analysis of NLR for PCOS prediction

be considered as women with PCOS have compensated the fasting glucose by secreting more insulin. Evaluation of the inflammatory markers revealed that Hs-CRP, NLR, and PLR were statistically significantly different when comparing the groups. Descriptive characteristics of the patients, including hormonal status, inflammatory markers, and comparison between groups are presented in Table 1.

Correlation analysis between PLR, NLR levels, and other clinical, biological laboratory markers revealed that there was no significant correlation between these parameters even in the control and PCOS groups. Evaluation of the correlation analysis is determined in Table 2.

The discriminative value of NLR for the presence of PCOS was evaluated using ROC curve analysis (Figure 1). The area under the ROC curve (AUC) was 0.643 (95% CI: 0.563-0.724, p<0.001) for NLR. The optimal cut-off value of NLR for detecting PCOS was ≥2.26, with a sensitivity of 57.6 % and a specificity of 62 %.

**Discussion**

Polycystic ovary syndrome seems like a benign disease, however, consequences like infertility and long-term complications such as diabetes mellitus and cardiovascular diseases, make PCOS a complex disease. In the studies, the prevalence has been stated as about 10%, however, women who suffered infertility experienced PCOS much more and vice versa [1, 12, 13]. Studies have been intensifying on the etiology of PCOS, and the treatment of infertility chiefly. Contemporary studies have exposed crucial progress about the pathway of the disease, though the proper cause of PCOS is still lacking. Obesity and peripheral insulin resistance are among the main risk factors and concomitant issues of PCOS [1, 9, 13]. Nonetheless, there was no significant difference in terms of BMI and waist circumference in the present study. The main factor in this circumstance was the adjusted BMI and age criteria of the study. The androgenic status of the patient is essential and even one of the diagnostic criteria. We investigated total testosterone in our study and it did not differ significantly, however, it is well known that free testosterone levels show the activity of androgenic status. In some studies, total testosterone was also similar to the control group, yet free testosterone was higher in women with PCOS [14, 15]. Impaired serum glucose homeostasis has been one of the most important and possible accused factors in PCOS, even in some types of the disease, its co-occurrence has not been shown [6]. Despite there was no significant difference between groups, women with PCOS had higher insulin resistance, which was examined with HOMA-IR in the present study, and this was similar to the literature [5, 9, 14]. The focused issue in the etiology of PCOS was the inflammatory process. Several markers of inflammation have been investigated and declared as possible diagnostic markers for PCOS [11, 16]. Complete blood count (CBC) parameters have started to be investigated as novel inflammatory markers. It is feasible and cheap, yet the main point was that most of the mediators have been secreted by lymphocyte, neutrophil, and platelets. Thus, the combination of these parameters like NLR and PLR might be precious markers for

diseases that progress with chronic inflammation. We have determined that PLR, NLR, and Hs-CRP have been differed statistically different. These markers were investigated in several studies. There was no consensus on the levels of Hs-CRP in PCOS. We have declared an increased level in patients with PCOS. Studies that determined similar levels of Hs-CRP comparing the controls and women with PCOS, have associated this result with obesity because obesity was one of the main sources of chronic inflammation [17, 18]. In the present study, the patients included in the control group were adjusted with the PCOS group in terms of BMI, and it was revealed that Hs-CRP differed significantly more. Most of the participants in the study were not obese. Thus, our study determined that Hs-CRP was higher in patients with PCOS independent of obesity.

Increased levels of neutrophil- to- lymphocyte ratio and platelet- to- lymphocyte ratio were the main findings of this study. That might be a shred of evidence that PCOS is related to chronic inflammation. Contemporary studies declared that NLR, PLR, and platelet-related parameters factors like Mean Platelet Volume (MPV) were related to the diseases based on chronic inflammation like systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, and Behcet's syndrome. Recent studies have stated that NLR and PLR were significantly higher in patients with PCOS than in healthy controls. Our findings were similar to them [16, 18-20]. As aforementioned, obesity could be a reason for this chronic inflammation, our study and Yilmaz et al have determined that elevated PLR and NLR were independent of high BMI values [18]. Pergialiotis et al achieved a valuable study that investigated the correlation of PLR and NLR with the metabolic and hormonal parameters, and concluded that these inflammatory markers were associated with follicle-stimulating hormone (FSH), free testosterone, androstenedione, sex hormone-binding globulin (SHBG) [20]. Our correlation analysis revealed that there was no relation between the parameters and PLR, NLR, including BMI, age, HOMA-IR, and testosterone levels. Besides this evidence, we aimed to evaluate NLR as a diagnostic marker for PCOS. The ROC analysis revealed that the optimal cut-off value of NLR for detecting PCOS was  $\geq 2.26$ , with a sensitivity of 57.6 % and a specificity of 62 %. Although the specificity and sensitivity were not appropriate for determining NLR as a diagnostic tool, the present study was one of the unique study that notice a value for diagnosis. Statistical analyses were not able to establish such a value for PLR and HsCrp in the present study.

The strength of the study was in its prospective design and well-organized participants' inclusion criteria. The limitation of the study was the lack of an elaborate hormonal status examination. The other limitation was the lack of separation of women in the PCOS group into subgroups in terms of different clinical characteristics. That study was one of the studies that expose the chronic inflammatory status and PCOS. The contemporary studies might be raised from that findings. To sum up, the present study has exposed that women with PCOS had a higher level of chronic inflammatory markers such as PLR, NLR, and Hs-CRP. However, the worth of this evidence is still missing, whether the increased inflammatory is related

to infertility or long- term complications like coronary heart diseases, or whether these markers could be used for treatment follow-up. Further studies might enlightened these questions in the near future.

#### Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

#### Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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#### Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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